FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
44342.013600	
U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR	
10/031650	

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JPOO/02940	9 May 2000	10 May 1999

TITLE OF INVENTION

COMPOSITIONS FOR RECOVERING HYPOFERTILITY

APPLICANT(S) FOR DO/EO/US

NIPPON SHINYAKU CO., LTD.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☐ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Copy of the first page of the published International Application Number WOOO/67768.

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR) 10/031650	INTERNATIONAL APPLICATION NO. PCT/JPOO/02940	ATTORNEY'S DOCKET NUMBER 44342.013600

21. The following fees are submitted:.	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :	
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO $970.00	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO $840.00	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $690.00	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $670.00	
☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $96.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$840.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	$0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	16 - 20 =	0	x $18.00	$0.00
Independent claims	8 - 3 =	5	x $78.00	$420.00
Multiple Dependent Claims **(check if applicable)**.			☐	$0.00
TOTAL OF ABOVE CALCULATIONS			=	$1,260.00
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**.			☐	$0.00
SUBTOTAL			=	$1,260.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	$0.00
TOTAL NATIONAL FEE			=	$1,260.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☒	$40.00
TOTAL FEES ENCLOSED			=	$1,300.00
			Amount to be: refunded	$
			charged	$

☐ A check in the amount of to cover the above fees is enclosed.

☒ Please charge my Deposit Account No. **50-1561** in the amount of **$1,300.00** to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **50-1561** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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31,900
REGISTRATION NUMBER

11/09/01
DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Hironori TOMI, et al.

Serial No.: T/B/A | Group Art Unit: T/B/A

Filed: Herewith | Examiner: T/B/A

For: COMPOSITIONS FOR RECOVERING HYPOFERTILITY

Commissioner for Patents and Trademarks
Box PCT
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Prior to the calculation of the filing fee and examination of the above-referenced new United States Patent Application, please amend the present Application as follows.

IN THE CLAIMS:

Please cancel claims 1-14 without prejudice, and replace them with new claims 15-30 as provided herein.

15. A composition for restoring compromised reproductive function comprising Withania somnifera Dunal.

16. A composition for restoring compromised reproductive function comprising an extract of Withania somnifera Dunal.

17. The composition of claim 15, wherein said composition restores reproductive function compromised by an endocrine disturbing chemical.

18. The composition of claim 16, wherein said composition restores reproductive function compromised by an endocrine disturbing chemical.

19. A food supplement for restoring compromised reproductive function comprising a food and Withania somnifera Dunal.

20. A food supplement for restoring compromised reproductive function comprising a food and an extract of Withania somnifera Dunal.

21. The food supplement of claim 19, wherein said food supplement restores reproductive function compromised by an endocrine disturbing chemical.

22. The food supplement of claim 20, wherein said food supplement restores reproductive function compromised by an endocrine disturbing chemical.

23. A method for preparing a composition for restoring compromised reproductive function comprising adding Withania somnifera Dunal as an active ingredient to a physiologically acceptable non-toxic and inert carrier.

24. A method for preparing a food supplement for restoring compromised reproductive function comprising adding to a food Withania somnifera Dunal as an active ingredient.

25. A method for preparing a composition for restoring compromised reproductive function comprising adding an extract of Withania somnifera Dunal as an active ingredient to a physiologically acceptable non-toxic and inert carrier.

26. A method for preparing a food supplement for restoring compromised reproductive function comprising adding to a food an extract of Withania somnifera Dunal as an active ingredient.

27. A method for restoring compromised reproductive function in a human subject comprising administering to the human subject an effective amount of the composition of claim 15.

28. A method for restoring compromised reproductive function in a human subject comprising administering to the human subject an effective amount of the composition of claim 19.

29. A method for restoring compromised reproductive function in a human subject comprising administering to the human subject an effective amount of the composition of claim 16.

30. A method for restoring compromised reproductive function in a human subject comprising administering to the human subject an effective amount of the composition of claim 20.

REMARKS

The present amendment has been made to delete multiple dependencies and otherwise bring the claims in conformance with United States patent practice, and to limit the fees. Early and favorable action is respectfully requested.

AUTHORIZATION

Please charge any required fee to the Greenberg Traurig Deposit Account No. 50-1561.

Respectfully submitted,
Greenberg Traurig, LLP

Date: November 9, 2001

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CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as express mail number EL 729389691 US in an envelope addressed to "Assistant Commissioner for Patents, Box PCT, Washington, D.C. 20231, on November 9, 2001.

David L. Heath

David L. Heath
Signature

11/9/01
Date of Signature

DESCRIPTION

COMPOSITIONS FOR RECOVERING HYPOFERTILITY

TECHNICAL FIELD

The present invention relates to Withania somnifera (Withania somnifera Dunal.) which is known to be a medicinal plant.

Withania somnifera is also known as ashwagondha (ashvaganda), sekitome-hozuki, winter cherry, asganh, asunda, asarna, phatalfoda, askandha, achubagandi, amucrang kalang, amukila, kilzang (all phonetic), and so on.

BACKGROUND ART

It has come to be known of late that endocrine disturbing chemicals (environmental hormones) existing in our living environment, such as bisphenol A, dibutyl phthalate, vinclozolin, polychlorobiphenyls, ethynylestradiol, nonylphenol, etc., not to speak of dioxins, affect the reproductive functions of animals to reduce their sexual activities either reversibly or at times irreversibly and impair male genital organs causing decreases in sperm count, in particular. These endocrine disturbing chemicals are present in the environment and act at low concentrations so that they

have become a social problem.

It is difficult, in the state of the art, to protect individuals from contaminations with such endocrine disturbing chemicals and all the countermeasures so far known are a negative measure which comprises measuring the concentrations of endocrine disturbing chemicals in foodstuffs and seeing to it that foods contaminated beyond tolerable concentration limits will be not ingested and a measure which comprises recommending the intake of diet fiber, chitin, chitosan, etc. which are expected to adsorb endocrine disturbing chemicals and let them be excreted as so adsorbed.

Meanwhile, Withania somnifera Dunal. is a tree of the genus Withania of the family Solanaceae, which is distributed in India and South Africa. It is a time-honored folk medicine or diet efficacious for sthenia, antirheumatism, antisenescence, and prophylaxis of marasmus in young children, among other indications (e.g. Kalpana Sharma and P. C. Dandiya; INDIAN DRUGS, 29 (6), 247-250) and, as such, has been used broadly.

As the constituents of Withania somnifera, alkaloids such as cuscohygrine, anahygrine, tropine, pseudotropine, anaferine, dl-isopellatierine, 3-tropyltigloate, withasomine, visamine, withaninine,

withanine, pseudowithaninine, 3-alpha-tigloyloxytropane, choline, etc. and withanolides such as withaferin A, sitoindosides I-X, withanolide N, withanolide O, withanolide D, withanolide E, withanolide P, withanolide S, withanolide Q, withanolide R, withanolide G, withanolide H, withanolide I, withanolide J, withanolide K, withanolide U, withanolide Y, etc. are known.

DISCLOSURE OF INVENTION

The object of the present invention is to redress or relieve the effects of *in vivo* contaminations with endocrine disturbing chemicals and, as such, provide a composition and a food for promoting recovery of the reproductive function compromised by such chemicals.

After their intensive research, the inventors of the present invention found that *Withania somnifera* has an action to promote recovery of compromised reproductive function and have perfected the present invention.

The present invention, therefore, encompasses a composition for restoring compromised reproductive function or a composition for redressing atrophic or impaired genital organs, characterized in that it comprises *Withania somnifera*, and a composition for restoring compromised reproductive function or a

composition for redressing atrophic or impaired genital organs, characterized in that it comprises an extract of Withania somnifera. Also encompassed is a compromised reproductive function-restorative composition or atrophic genital organ-redressing composition for restoration of the reproductive function compromised by endocrine disturbing chemicals.

Stated differently, the invention is concerned with the use of Withania somnifera for the production of a composition comprising Withania somnifera as the active ingredient for restoring compromised reproductive function; the use of Withania somnifera for the production of a composition comprising an extract of Withania somnifera as the active ingredient for restoring compromised reproduction function; a method of restoring compromised reproductive function which comprises giving a composition comprising Withania somnifera to an individual, and a method of restoring compromised reproductive function which comprises giving a composition comprising an extract of Withania somnifera to an individual.

In the present invention, Withania somnifera can be used regardless of whether it is a dried one or an undried one. And coarse cuttings or pulverizates of its

root, leaf or whole plant can be orally taken or ingested as such or together with drinking matter such as water, lukewarm water, a fruit juice or milk. Alternatively, it can be judiciously extracted with hot water or an alcohol and the extract taken orally or ingested.

The extract of Withania somnifera can be obtained by treating fragments of the root, leaf or whole plant of Withania somnifera with a suitable extractant, such as water (hot water) or an alcohol, and subjecting the extract to concentration, optionally to dryness. This extract is preferably one containing not less than 1.0 weight % of alkaloids and not less than 1.0 weight % of withanolides, more preferably not less than 1.2 weight % of alkaloids and not less than 1.4 weight % of withanolides. The extract can be taken orally or ingested as it is or as suspended or dissolved in drinking matter such as water, lukewarm water, a fruit juice, tea or milk.

The dosage, ingestion amount or decoction amount (when a decocta is to be taken orally or ingested) of Withania somnifera for restoring compromised reproductive function is dependent on the recipient's sex and age, health status, and target organ or site but may appropriately be within the range of generally 1~100 g, preferably 2~20 g, as dry Withania somnifera

per day per adult human. In the case of an extract, the daily amount per adult human is generally in the range of 0.1~10 g, preferably in the range of 0.2~5 g. In any event, the extract can be taken orally or ingested once daily or in 2~4 divided doses a day. The intake or ingestion time is not particularly restricted but may for example be before a meal, between meals, after a meal, or at bedtime. The composition can be taken orally or ingested together with a food.

The compromised reproductive function-restorative composition of the invention (hereinafter referred to as the composition of the invention) may be Withania somnifera or an extract thereof as such or a composition containing Withania somnifera, for example in the range of 0.01% ~ 99.5%, preferably 0.5% ~ 90%, in a physiologically acceptable, nontoxic and inert carrier.

As the carrier, a solid, semisolid or liquid diluent, a filler and one or more other formulation additives can be mentioned. The composition of the invention may be provided in any form such as neat powders, capsules, tablets, sugar-coated tablets, granules, powders, suspensions, solutions, syrups and drops, among others. Depending on cases, injectable forms may be employed.

The composition of the invention is useful for promoting recovery of compromised reproductive function in animals inclusive of man, particularly recovery of reproductive function in males. Furthermore, the composition of the invention is recommendable for promoting recovery of atrophic or impaired male genital organs. Therefore, the composition of the invention can be used in the field of medicine as a therapeutic or prophylactic drug.

In addition, the composition of the invention can be added to foods, namely general foods such as curry, pilaf, prepared dishes, etc. or other foods inclusive of drinks and cakes, or provided in such forms as tablets, capsules or granules for use as the so-called nutritional supplement or health food. Therefore, a compromised reproductive function-restorative food or atrophic genital organ-redressing food characterized by comprising Withania somnifera, a compromised reproductive function-restorative food or atrophic genital organ-redressing food characterized by comprising an extract of Withania somnifera, and such a compromised reproductive function-restorative food or atrophic genital organ-redressing food for restoring the reproductive function compromised by endocrine disturbing chemicals also fall within the scope of the

present invention.

Stated differently, the above aspects of the invention are concerned with the use of Withania somnifera for the production of foods containing Withania somnifera as the active ingredient for restoring compromised reproductive function, the use of Withania somnifera for the production of foods containing an extract of Withania somnifera as the active ingredient for restoring compromised reproductive function, a method of restoring compromised reproductive function which comprises giving a food containing Withania somnifera to an individual, and a method of restoring compromised reproductive function which comprises giving a food containing an extract of Withania somnifera to a living body.

BEST MODE FOR CARRYING OUT THE INVENTION

The following example and test examples illustrate the present invention in further decaudal epididymis.

Example 1

Preparation of an extract

Ten (10) kg of the dried root of Withania somnifera Dunal. was washed thoroughly with water and, after drying, crushed into small pieces about 2~5 mm in diameter. To these pieces was added 10 volumes of 50%

ethanol and an extraction was carried out under reflux at 60°C for 4 hours. The resulting extract was concentrated to dryness under reduced pressure to give 50 g of a dry extract of Withania somnifera. Compositional analysis of this extract by HPTLC in accordance with the literature (BHATTACHARYA S. K. et al., PHYTOTHERAPY RESEARCH, 9, 110~113 (1995)) revealed that the total alkaloid content was 1.70 weight % and the withanolides content was 1.98 weight %.

Test Example 1

Compromised reproductive function-restoring effect (1)

SD rats aged 11 weeks (in groups of 8) were orally dosed with 3 mg/kg of the endocrine disturbing chemical ethynylestradiol suspended in 0.5% sodium carboxymethylcellulose (CMC) solution (ethynylestradiol 0.6 mg/mL) or, as control, 5 mL/kg of 0.5% CMC solution once daily in the morning for 2 weeks, and after the administration course, the testis, epididymis, prostate and seminal vesicle were respectively weighed. The results are shown in Table 1.

Table 1

	Testis	Epididymis	Seminal vesicle	Prostate
CMC-dosed group	836.1 ±51.3	250.9 ±13.8	343.8 ±35.9	212.7 ±39.3
Ethynylestradiol-dosed group	705.4* ±67.9	117.5* ±7.5	88.1* ±23.7	77.4* ±17.6

*: p<0.05 (Student's t-test), n=8 (mg/100 gBW)

It is clear from Table 1 that the rat genital organs atrophied owing to the influence of the endocrine disturbing chemical.

Then, the above rats (in groups of 8) with the reproductive function compromised by the endocrine disturbing chemical were orally dosed with 5 mL/kg of 2% gum arabic solution or either 100 mg/kg (Withania somnifera 20 mg/mL) or 500 mg/kg (Withania somnifera 100 mg/mL) of the dry extract of Withania somnifera according to Example 1 as suspended in 2% gum arabic solution once daily in the morning for 2 weeks, and the degrees of recovery of reproductive function due to Withania somnifera were evaluated. The results are shown in Table 2.

Table 2

	Testis	Epididymis	Seminal vesicle	Prostate
Gum arabic-dosed group	596.8 ±86.6	119.5 ±15.1	171.5 ±65.9	116.2 ±28.9
Withania somnifera 100 mg/kg-dosed group	581.1 ±60.2	116.2 ±10.7	229.7* ±30.2	131.4 ±15.8
Withania somnifera 500 mg/kg-dosed group	618.5 ±97.8	124.7 ±13.3	207.7 ±44.3	123.8 ±29.2

*: p<0.05 (Dunnett t-test), n=8 (mg/100 gBW)

It is clear from Table 2 that the group dosed with Withania somnifera showed a recovery of the genital organs, particularly the seminal vesicle and prostate, which had atrophied owing to the influence of the

endocrine disturbing chemical.

Rat husbandry conditions: room temperature 21~25°C, humidity 45~60%, artificial lighting 12 hrs (7:00 a.m. ~ 7:00 p.m.), ventillation frequency 15/hr, solid food (CE-2, CLEA Japan Inc.) and drinking water ad libitum.

Test Example 2

Compromised reproductive function-restoring effect (2)

Slc:SD rats aged 10 weeks (in groups of 10) were orally dosed with 3 mg/kg of the endocrine disturbing chemical ethynylestradiol suspended in 0.5% sodium carboxymethylcellulose (CMC) solution (ethynylestradiol 0.3 mg/mL) or, as control, 10 mL/kg of 0.5% CMC solution once daily in the morning for 10 days, and at 1 week after the administration course, the testis, epididymis, prostate and seminal vesicle were respectively weighed. The results are shown in Table 3.

Table 3

	Testis	Epididymis	Seminal vesicle	Prostate
CMC-dosed group	863.1 ±63.5	246.9 ±18.4	337.5 ±28.4	203.6 ±20.7
Ethynylestradiol-dosed group	731.3* ±53.6	154.5* ±21.0	170.0* ±33.1	102.3* ±19.9

*: $p<0.05$ (Student's t-test), n=10 (mg/100 gBW)

It is apparent from Table 3 that the rat genital organs atrophied under the influence of the endocrine

disturbing chemical.

Then, the above rats with the reproductive function compromised by the endocrine disturbing chemical (in groups of 10) were orally dosed with 10 mL/kg of 2% gum arabic solution or 100 mg/kg of the dry extract of Withania somnifera according to Example 1 as suspended in 2% gum arabic solution (Withania somnifera 10 mg/mL) once daily in the morning for 4 weeks, and the degrees of recovery of reproductive function due to Withania somnifera were evaluated. The results are shown in Table 4.

Table 4

	Testis	Epididymis	Seminal vesicle	Prostate
Gum arabic-dosed group	781.2 ±100.9	195.7 ±22.3	317.9 ±26.7	204.7 ±30.8
Withania somnifera 100 mg/kg-dosed group	799.1 ±60.2	222.1* ±18.3	326.6 ±52.6	239.3 ±47.9

*: p<0.05 (Dunnett t-test), n=10 (mg/100 gBW)

It is apparent from Table 4 that compared with the control group (gum arabic-dosed group), the Withania somnifera-dosed group showed an accelerated recovery of the atrophic or impaired genital organs caused by the endocrine disturbing chemical, with a significant difference for the epididymis.

Rat husbandry conditions: room temperature 21~25°C, humidity 45~60%, artificial lighting 12 hrs (7:00 a.m.

~ 7:00 p.m.), ventilation frequency 15/hr, solid food (CE-2, CLEA Japan Inc.) and drinking water ad libitum.

Test Example 3

Sperm count and motile sperm rate

Using rats with the genital organs impaired by ethynylestradiol as in Test Example 2, the sperm count and motile sperm rate were investigated. The results are shown in Table 5.

Table 5

	Caudal epididymis (weight, g)	Sperm count ($\times 10^6$)	Sperm count /caudal epididymis ($\times 10^6$)	Sperm count/ epididymis ($\times 10^6$)	Motile sperm rate (%)
CMC-dosed group	0.190 ±0.017	110.3 ±18.1	580.6 ±79.0	565.2 ±81.0	74.7 ±8.0
Ethynylestradiol-dosed group	0.076 ±0.011	13.0* ±12.7	160.2* ±147.2	88.8* ±87.4	40.6* ±30.0

*: $p<0.05$ (Student's t-test), n=10

Then, the above rats with the reproductive function compromised by the endocrine disturbing chemical (in groups of 10) were orally dosed with 10 mL/kg of 2% gum arabic solution or 100 mg/kg of the dry extract of Withania somnifera according to Example 1 as suspended in 2% gum arabic solution (Withania somnifera 10 mg/mL) once daily in the morning for 4 weeks and, after the administration course, the sperm count and motile sperm rate in each rat were determined by the following methods.

(1) Method for determination of motile sperm rate

From the right epididymis, the caudal epididymis was excised and weighed with Sartorius electronic balance LC620-S. The caudal epididymis was placed in a sperm collection vial containing 5 mL of BSA-Hanks solution and cut 3 times to cause the sperm to swim out. A 0.05 mL portion of the sperm fluid was sampled and diluted with 0.95 mL of BSA-Hanks solution for use as a diluted sperm fluid. The number of non-motile sperms in the diluted sperm fluid was determined with Thoma's hemocytometer. After this counting of non-motile sperms, the vessel containing the diluted sperm fluid was immersed in hot water and, after return to room temperature, the sperms were counted with the hemocytometer. When the sperm population in the sperm fluid was found to be small (low turbidity) by gross observation, an aliquot of the fluid was taken as a motile sperm counting sample fluid and the above measurement was carried out. Using the measured values, the motile sperm rate was calculated by means of the following equation [1].

Motile sperm rate (%) =

(number of sperms - number of non-motile sperms)/ number of sperms × 100 [1]

(2) Method for determination of sperm count

The caudal epididymis in the sperm collection vial used in the above procedure (1) was further cut to release sperms and the fluid in the vial was filtered through a nylon-mesh sieve. The stock filtrate, 0.1 mL, was diluted with 1.9 mL of formalinized saline and the number of sperms was determined with Thoma's hemocytometer. When the sperm population in the sperm sample was considered to be too small (low turbidity), the stock sperm fluid was not diluted but the vessel was directly immersed in hot water and, after reutrn to room temperature, the number of sperms was determined. Moreover, the number of sperms (sperm count) per caudal epididymis was calculated using the number of sperms determined and the dilution factor by means of the equation given below, with the value per caudal epididymis unit weight (g) being taken as the sperm count/caudal epididymis and the value per epididymis as the sperm count/epididymis.

Sperm count = measured number of sperms × dilution factor

Number of sperms/caudal epididymis =

number of sperms determined × dilution factor/

weight of the caudal epididymis epididymis (g)

Number of sperms/epididymis =

(number of sperms/caudal epididymis) × weight of epididymis (g)

The results of the above test are shown in Table 6.

Table 6

	Caudal epididymis (weight, g)	Sperm count ($\times 10^6$)	Sperm count /caudal epididymis ($\times 10^6$)	Sperm count/ epididymis ($\times 10^6$)	Motile sperm rate (%)
Gum arabic-dosed group	0.153 ±0.024	78.4 ±34.8	493.4 ±180.9	437.2 ±185.9	55.9 ±19.7
Withania somnifera 100 mg/kg-dosed group	0.172 ±0.022	101.4 ±39.4	577.6 ±171.1	541.5 ±187.4	57.1 ±19.4

*: $p<0.05$ (Dunnett t-test), n=10

It is apparent from Table 6 that in the sperm count and motile sperm rate depressed by the endocrine disturbing chemical, too, early recoveries were obtained as compared with control.

CLAIMS

1. A compromised reproductive function-restorative composition characterized in that it comprises Withania somnifera Dunal.

2. A compromised reproductive function-restorative composition characterized in that it comprises an extract of Withania somnifera Dunal.

3. A compromised reproductive function-restorative composition according to Claim 1 or 2 for restoring the reproductive function compromised by an endocrine disturbing chemical.

4. A compromised reproductive function-restorative food characterized in that it comprises Withania somnifera Dunal.

5. A compromised reproductive function-restorative food characterized in that it comprises an extract of Withania somnifera Dunal.

6. A compromised reproductive function-restorative food according to Claim 4 or 5 for restoring the reproductive function compromised by an endocrine disturbing chemical.

7. Use of Withania somnifera Dunal. for the production of a composition comprising Withania somnifera Dunal. as the active ingredient for restoring

compromised reproductive function.

8. Use of Withania somnifera Dunal. for the production of a food comprising Withania somnifera Dunal. as the active ingredient for restoring compromised reproductive function.

9. Use of Withania somnifera Dunal. for the production of a composition comprising an extract of Withania somnifera Dunal. as the active ingredient for restoring compromised reproductive function.

10. Use of an extract of Withania somnifera Dunal. for the production of a food comprising an extract of Withania somnifera Dunal. as the active ingredient for restoring compromised reproductive function.

11. A method of restoring compromised reproductive function which comprises giving a composition comprising Withania somnifera Dunal. to an individual.

12. A method of restoring compromised reproductive function which comprises giving a food comprising Withania somnifera Dunal. to an indivisual.

13. A method of restoring compromised reproductive function which comprises giving a composition comprising an extract of Withania somnifera Dunal. to an individual.

14. A method of restoring compromised

reproductive function which comprises giving a food comprising an extract of Withania somnifera Dunal. to an individual.

Abstract

The object of the present invention is to redress or relieve the effects of *in vivo* contaminations with endocrine disturbing chemicals and, as such, provide a composition and a food for promoting recovery of the reproductive function compromised by such chemicals.

The present invention encompasses a composition for restoring compromised reproductive, characterized in that it comprises *Withania somnifera*.

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Declaration and Power of Attorney for Patent Application

特許出願宣言書及び委任状

Japanese Language Declaration

日本語宣言書

私は、以下に記名された発明者として、ここに下記の通り宣言する：

As a below named inventor, I hereby declare that

私の住所、郵便の宛先そして国籍は、私の氏名の後に記載された通りである。

My residence, post office address and citizenship are as stated next to my name.

下記の名称の発明について、特許請求範囲に記載され、且つ特許が求められている発明主題に関して、私は、最初、最先且つ唯一の発明者である（唯一の氏名が記載されている場合）か、或いは最初、最先且つ共同発明者である（複数の氏名が記載されている場合）と信じている。

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Compositions For Recovering

Hypofertility

上記発明の明細書はここに添付されているが、下記の箱がチェックされている場合は、この限りでない：

the specification of which is attached hereto unless the following box is checked:

is filed concurrently herewith

☐ _____ の日に出願され、
この出願の米国出願番号またはＰＣＴ国際出願番号は、
_____ であり、且つ
_____ の日に補正された出願（該当する場合）

☐ was filed on _____
as United States Application Number or
PCT International Application Number
_____ and was amended on
_____ (if applicable).

私は、上記の補正書によって補正された、特許請求範囲を含む上記明細書を検討し、且つ内容を理解していることをここに表明する。

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

私は、連邦規則法典第３７編規則１．５６に定義されている、特許性について重要な情報を開示する義務があることを認める。

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

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私は、ここに、以下に記載した外国での特許出願または発明者証の出願、或いは米国以外の少なくとも一国を指定している米国法典第３５編第３８５条(a)によるＰＣＴ国際出願について、同第１１９条(a)-(d)項又は第３６５条(b)項に基づいて優先権を主張するとともに、優先権を主張する本出願の出願日よりも前の出願日を有する外国での特許出願または発明者証の出願、或いはＰＣＴ国際出願については、いかなる出願も、下記の枠内をチェックすることにより示した。

I hereby claim foreign priority under Title 35, United States Code, Section 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT international application having a filing date before that of the application for which priority is claimed

Prior Foreign Application(s) 外国での先行出願			Priority Not Claimed 優先権主張なし
Hei-11/128,335	Japan	10/5/99	☐
(Number) (番号)	(Country) (国名)	(Day/Month/Year Filed) (出願日／月／年)	
			☐
(Number) (番号)	(Country) (国名)	(Day/Month/Year Filed) (出願日／月／年)	

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I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

(Application No.) (出願番号)	(Filing Date) (出願日)	(Application No.) (出願番号)	(Filing Date) (出願日)

私は、ここに、下記のいかなる米国出願についても、その米国法典第３５編第１２０条に基づく利益を主張し、又米国を指定するいかなるＰＣＴ国際出願についても、その同第３８５条(c)に基づく利益を主張する。また、本出願の各特許請求の範囲の主題が、米国法典第３５編第１１２条第１段に規定された態様で、先行する米国出願又はＰＣＴ国際出願に開示されていない場合においては、その先行出願の出願日と本国内出願日またはＰＣＴ国際出願日との間の期間中に入手された情報で、連邦規則法典第３７編規則１．５６に定義された特許性に関わる重要な情報について開示義務があることを承認する。

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(Application No.) (出願番号)	(Filing Date) (出願日)	(Status: Patented, Pending, Abandoned) (現況：特許許可、係属中、放棄)
(Application No.) (出願番号)	(Filing Date) (出願日)	(Status: Patented, Pending, Abandoned) (現況：特許許可、係属中、放棄)

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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書類送付先	Send Correspondence to: Eugene C. Rzucidlo, Esq. Reg. No. 31,900 GREENBERG TRAURIG, LLP 885 Third Avenue, 21st Floor New York, New York 10022
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（第三以下の共同発明者についても同様に記載し、署名をすること）	(Supply similar information and signature for third and subsequent joint inventors.)

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直通電話連絡先：（氏名及び電話番号）	Direct Telephone Calls to: (name and telephone number)

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住所	Residence
国籍	Citizenship
郵便の宛先	Post Office Address

（第三以下の共同発明者についても同様に記載し、署名をすること）

(Supply similar information and signature for third and subsequent joint inventors.)